COLOR FORMATION IN COOKED MODEL AND MEAT SYSTEMS WITH ORGANIC AND INORGANIC COMPOUNDS

INTRODUCTION

ONE OF THE functions of nitrite salts in curing meat products is the formation of the characteristic red color. Recently, however, objections have been raised to the use of nitrite for this purpose because of the possible implication of this salt in the formation of potential carcinogens. Elimination of nitrite from cured meat products results in the beige or tan color of hemochrome, the ferrous form of denatured myoglobin and hemoglobin. Substitutes for nitrite in producing the desirable color have been reported in journals and the patent literature. Tarladgis (1967) claimed a number of nitrogenous heterocyclic compounds produced red color in cured meats. Howard et al. (1973) studied color formation by nicotinic acid esters and amides in a model system and in a ground meat mixture. They confirmed that ascorbic acid or glucono-Δ-lactone improved color. Although they reported varying rates of fading in model systems, Howard et al. (1973) did not relate these rates to color stability in ground meat. Dekker (1958), Coleman and Steffen (1949, 1951), Hopkins and Sato (1971), Van den Oord and DeVries (1971), and Bernholdt and Roschen (1971) reported a wide variety of nitrogenous bases maintained color in fresh meat and/or cured meats. Hopkins and Sato (1971) and Van den Oord and DeVries (1971) claimed the effect was due to complex formation of the compounds with the heme pigment. Studies to date have been restricted, principally, to reagents forming coordinate covalent (low spin) complexes with heme pigments (Akoyunoglou et al., 1963; Howard et al., 1973). Such a restriction, however, may not be necessary, or even possible, depending on the type of pigment formed or ligand used.

We have surveyed a large number of compounds, principally various classes of nitrogen-containing material, to determine whether substitutes for nitrite could be found that would produce the stable, characteristic color of cured meat by complex formation with the heme pigments or by reaction with other meat components. The results of the investigation are reported herein.

MATERIALS & METHODS

BEEF, PORK and pork fat, stored in Cryovac bags at −12°C for periods of time ranging from 1 wk to 3 months, were thawed overnight at 8°C and used to prepare emulsions according to Fox et al. (1967) with all of the cure ingredients except sodium nitrite. The same type of emulsion was used for studies with frankfurters and in a model test tube system. For the test tube studies, emulsion was mixed with twice its weight of distilled water and homogenized in a Waring Blendor for about 90 sec to obtain a slurry. Reference samples were prepared by adding 0.02% sodium nitrite and 0.05% ascorbic acid to 5g of slurry in a test tube. Test samples were prepared in a similar manner, substituting 0.1–0.2% of the particular compound under study for the sodium nitrite. The slurries were mixed with a spatula and heated at 70°C for 45–50 min. After 5–10 min of heating, the slurries were mixed again to secure homogeneous distribution. Compounds producing an acceptable color in the slurries in the test tubes were tested in frankfurters.

The colors produced in both test tube and frankfurter systems were evaluated visually under fluorescent light by a group of three to five members of the laboratory by comparison with color produced by sodium nitrite in the reference sample.

The test compounds screened for color formation were obtained from commercial sources or were synthesized in the laboratory by conventional procedures. The chemicals were purified by recrystallization or redistillation as needed.

RESULTS & DISCUSSION

MORE THAN 300 compounds, in various classes of chemicals, were tested for their ability to form hemochromes with meat slurries and emulsions at 70°C. The largest number were nitrogen-containing compounds. A listing of many of these substances, and the color of hemochrome formed in meat slurry, are given in Table 1. The most effective color forming compounds were found among the substituted pyridines and isoquinolines. Color formation seems to be related to the nature of the substituent and its position on the ring. The best color was produced by pyridine compounds containing carbonyl groups. Carboxy groups, however, reduced the effectiveness of color formation even when isolated from the pyridine ring by a methylene group; the slight pink pigment produced was difficult to differentiate from the trace of pink color produced by ascrobate alone.

The 3- and 4-carboxypyridines as well as 4-hydroxypyridine are ionized at the pH of the meat slurry and emulsion (Roberts and Caserio, 1964) and this may account for the lack of complex formation. The corresponding 3- and 4-esters and amides, which do not ionize, formed hemochromes. In general, 2-substituted carbonyl pyridines yielded a tan or beige color that was similar to that of the slurry without nitrite. These derivatives do not form hemochromes at the concentrations studied, possibly due to steric hindrance preventing formation of the nitrogen-iron bond. The 3-substituted carbonyl pyridines produced a pink to reddish coloration, similar to that of the nitrite containing controls, and the carbonyl pyridines substituted in the 4-position produced slurries with pigments of various shades of pink and purple. Aldehyde, alkyl, amine, acyl and hydroxy derivatives generally behaved in a similar fashion. Fox et al. (1974a) studied the spectra of these heme complexes and

discussed the relationship of the colors produced to the energy levels of the bonding and antibonding orbitals of the π electrons of the aromatic ring.

The quinoline compounds tested did not form colored complexes with myoglobin in the meat slurries but isoquinolines, for the most part, yielded hemochromes. However, isoquinoline substituted in the 1 or 3 position, adjacent to the nitrogen, did not form a complex with heme. The inability to form a hemochrome may be due to steric hindrance as noted for the 2-substituted pyridines.

Table 1—Nitrogenous heterocyclic compounds tested for color production in meat slurries heated at 70°C for 45 min

Compound	Color	Compound	Color
Pyridine	Pink	**Aminopyridines**	
Acids, amides, esters		2-aminopyridine	Tan
2-carboxypyridine	Tan	3-aminopyridine	Beige
3-carboxypyridine	Beige	4-aminopyridine	Beige
4-carboxypyridine	Beige	2-aminomethylpyridine	Tan
2-(3-pyridyl)acetic acid	Beige	3-aminomethylpyridine	Tan
3-pyridylsulfonic acid	Beige	4-aminomethylpyridine	Tan
3-carboxymethylpyridine	Pink	2-amino-3-methylpyridine	Beige
3-carboxyethylpyridine	Pink	2-amino-4-methylpyridine	Tan
3-carboxypropylpyridine	Pink	2-amino-5-methylpyridine	Tan
3-carboxybutylpyridine	Pink	2-amino-6-methylpyridine	Beige
3-carboxyhexylpyridine	Pink	**Pyrroles**	
4-carboxymethylpyridine	Pink	Pyrrole	Beige
4-carboxyisopropylpyridine	Pink	N-methylpyrrole	Beige
4-carboxyisopentylpyridine	Pink	3-methylpyrrole	Beige
3-amidopyridine	Pink	N-methyl-3-formylpyrrole	Beige
3-(N-ethyl)amidopyridine	Pink	3-pyrroline	Beige
isonicotinic acid hydrazide[a]	Pink	Pyrrolidine	Beige
Aldehydes		N-(β-dihydroxyethyl)-3,4-dicarboxymethylpyrrolidine	Beige
2-formylpyridine	Beige	**Quinolines**	
3-formylpyridine	Pink	Quinoline	Beige
4-formylpyridine	Purple	2-aminoquinoline	Beige
2-pyridyloxime	Beige	5-hydroxyquinoline	Beige
Acylpyridines		8-hydroxyquinoline	Beige
2-acetylpyridine	Beige	isoquinoline	Purple
3-acetylpyridine	Purple	4-amino-isoquinoline	Purple
4-acetylpyridine	Purple	5-amino-isoquinoline	Orange
2-propionylpyridine	Tan	4-acetyl-isoquinoline	Purple
3-propionylpyridine	Purple	4-bromo-isoquinoline	Pink
4-propionylpyridine	Purple	5-bromo-isoquinoline	Brown
2-butyroylpyridine	Tan	1-carboxy-isoquinoline	Tan
3-butyroylpyridine	Pink	4-carboxy-isoquinoline	Purple
4-butyroylpyridine	Purple	4-cyano-isoquinoline	Purple
2-benzoylpyridine	Tan	1-hydroxy-isoquinoline	Beige
3-benzoylpyridine	Purple	5-hydroxy-isoquinoline	Pink
4-benzoylpyridine	Tan	3-methyl-isoquinoline	Tan
Hydroxypyridines		4-nitro-isoquinoline	Beige
2-hydroxypyridine	Tan	5-nitro-isoquinoline	Purple
3-hydroxypyridine	Pink	5-sulfonic-isoquinoline	Beige
4-hydroxypyridine	Tan	**Miscellaneous**	
Alkylpyridines		3,5-dichloropyridine	Beige
2-methylpyridine	Tan	2,2'-dipyridyl	Tan
3-methylpyridine	Beige	2,2'-dipyridylamine	Tan
4-methylpyridine	Beige	Myosmine	Tan
2-ethylpyridine	Tan	pyridine-N-oxide	Beige
3-ethylpyridine	Pink	N-methylpyridine iodide	Beige
4-ethylpyridine	Tan	N-ethylpyridine iodide	Beige
2,4-dimethylpyridine	Tan	Trigonelline	Beige
2,6-dimethylpyridine	Tan	2,4,6-tripyridyl-5-triazine	Beige
3,4-dimethylpyridine	Tan	3-cyanopyridine	Pink
3,5-dimethylpyridine	Tan		
2,4,6-trimethylpyridine	Tan		
2-vinylpyridine	Tan		
4-vinylpyridine	Pink		

[a] Isoniazid

The 3- and 4-acyl substituted pyridines produced the most stable hemochromes in the slurry; therefore, they were tested for their ability to form color in frankfurters. The results are shown in Table 2. On the basis of preliminary experiments, a series of 3-substituted acyl pyridines with increasing side chain length were either purchased or synthesized and also investigated for their ability to form a hemochrome in frankfurter emulsions. A semiquantitative, subjective evaluation of pigment stability in air was made by observing the color immediately and 20 min after exposing a fresh surface of the frankfurter. Pyridine itself gave a pink hemochrome that faded almost completely after 20 min exposure to air. The pigment produced by the nitrite control faded slightly. 3-Acetyl- and 3-propionylpyridines produced a purplish-pink pigment while the remaining 3-acyls, from butyroyl to capryloyl, were pink. The pink pigments, in general, were less stable than the purplish complexes, fading to some extent on exposure to air. 3-Butyroylpyridine, however, produced a good pink pigment which showed the same stability as the nitrite control.

A number of compounds have been reported in the literature to form hemochrome complexes in fresh and cured meat. Some of these were tested for their ability to form colored complexes in frankfurters (Table 2). Nicotinic acid and the methyl and hexyl esters formed tan, light pink, and pink complexes, respectively, which were less stable to air than the nitrite control. Substituted purines and pyrimidines formed no colored complexes with myoglobin, although pyrimidine itself produced an unstable pink color which faded completely on exposure to air. The purine and pyrimidine derivatives have substituents α to the nitrogen atoms of the pyrimidine ring, thus steric hindrance may account for their failure to produce colored pigments.

The diazines pyridazine, pyrimidine, and pyrazine contain two ring nitrogen atoms α, β and γ to each other. The color of the hemochromes produced by these compounds is analogous to those formed by the 2-, 3- and 4-substituted pyridines: α-diazine (pyridazine) formed a tan, or beige, pigment; β-diazine (pyrimidine) a pink hemochrome; and the γ-diazine (pyrazine) a purple complex. Imidazole produced a frankfurter with a good, stable pink color, but of the substituted imidazoles tested only histidine yielded a slightly pink pigment.

Triazine, tetrazole, and several other reportedly effective compounds produced

either no color or very weak hemochromes under our experimental conditions. While we have no explanation for the failure of these compounds to form color under what are otherwise favorable conditions, we have noticed variations in the intensity of pigment produced by any given compound in frankfurter emulsions made with different lots of meat.

Only eight compounds in Table 2 can be considered to produce pigments stable to exposure to air for at least 20 min. The group consisting of 3- and 4-acetylpyridine, 3-propionylpyridine and imidazole were somewhat more effective than 3-butyroylpyridine, pyrazine, 4-formylpyridine and nitrite.

In view of the pigment formation by histidine the effect of a series of amino acids and the amines, taurine and histamine, were investigated. The production of hemochrome by these compounds (Table 3), was not uniform and the intensity of the pinkish color, when it was produced, was difficult to differentiate from that produced in the controls containing ascorbate only. Since the amino acids and the two amines do not form denatured globin hemochrome complexes, the effect of their presence may be to protect and stabilize the pigment against complete denaturation as observed spectrally for pyridine derivatives (Fox et al., 1974b). They found that reflectance absorption spectra of the cut surfaces of several pyridine-containing frankfurters had peaks at 540 and 580 nm indicating the presence of oxymyoglobin, a result of incomplete denaturation of the pigment.

The use of ascorbate or cysteine alone, without nitrite or a test substitute for nitrite, led to the formation of a pinkish tinge in frankfurter or emulsion slurry. A number of reductants were tested in these systems. The results in Table 4 show that only stannous chloride produced a hemochrome in these systems in the absence of nitrite.

A series of cyano derivatives were studied for pigment formation. The results, shown in Table 5, confirm the con-

Table 2—Nitrogenous heterocyclic compounds tested for color production in frankfurters

Compound	Color	Fading[a]
Pyridine	Pink	Almost complete
Nicotinic acid	Tan	—
Methylnicotinate	Light pink	Some
Hexylnicotinate	Pink	Much
3-formylpyridine	Pink	Much
4-formylpyridine	Purplish-pink	Slight
3-Acetylpyridine	Purplish-pink	None
4-Acetylpyridine	Purplish-pink	None
3-propionylpyridine	Purplish-pink	None
3-butyroylpyridine	Pink	Slight
3-valeroylpyridine	Pink	Much
3-caproylpyridine	Pink	Much
3-heptoylpyridine	Pink	Much
3-capryloylpyridine	Pink	Some
Trigonelline	Tan	—
p-aminobenzoic acid	Tan	—
m-aminobenzoic acid	Tan	—
Triazine	Tan	—
Tetrazole	Grey	—
Pyrrole	Tan	—
Purine	Tan	—
Adenine	Tan	—
Guanine	Tan	—
Xanthine	Tan	—
Uric acid	Tan	—
Pyrimidine	Pink	Complete
Thymine	Tan	—
Cytosine	Tan	—
Uracil	Tan	—
Pyrazine	Purple	Slight
Pyrazinamide	Purple	Slight
Pyridazine	Tan	—
Imidazole	Pink	None
4-imidazole carboxylic acid	Tan	—
Histidine	Sl. pink	None
Histidine, N-acetyl	Sl. pink	None
Histamine	Tan	—
Histamine, N-acetyl	Tan	—

[a] Degree of fading on 20 min exposure to air. Slight fading observed with nitrite.

Table 3—Effect of some amino acids on pigment formation in meat slurries and frankfurters

Amino acids	Color	Fading
Arginine	Pink	1 hr
Asparagine	Tan	—
Cysteine	Beige	15—20 min
Glutamic acid	Tan	—
Lysine	Tan	—
Methionine	Yellowish-tan	—
Proline	Beige	
Tryptophane	Beige	
Tyrosine	Beige	
Proline	Beige	
Proline, ethyl ester	Beige	
Taurine	Beige	

Table 4—The effect of antioxidants and reductants on pigment formation in meat slurries and frankfurters

Compound	Color
Hydrindantin	Brown
5-OH-1,4-naphthothioquinone	Brown
Kojic acid	Beige
Methylhydroquinone	Beige
Xanthydrol	Beige
2-Methyl-1,4-naphthoquinone	Beige
Quinhydrone	Beige
Stannous chloride	Pink
Ferrous carbonyl	Beige
Ferrous chloride	Beige
Cysteine[a]	Beige
Ascorbate[a]	Beige

[a] Coleman, Steffen and Hopkins, 1951, U.S. Pat. 2,541,572, have claimed ascorbic acid alone in fresh meat.

Table 5—The effect of cyanides, isocyanides and isocyanates on pigment formation in meat slurries and frankfurters

Compound	Color	Fading
n-butylisocyanide	Orangish-pink	None
benzylisocyanide	Orangish-pink	None
n-butylcyanide	Tan	—
benzylcyanide	Tan	—
p-chlorophenylcyanide	Tan	—
phenylcyanide	Tan	—
p-nitrophenylisocyanate	Tan	—
p-phenylazophenylisocyanate	Tan	—

cept that iron complex formation with cyano groups is through the carbon and not the nitrogen atom (Pauling, 1960). Thus the isocyanides yielded orangish-pink pigments while the organic cyanides and isocyanates were unable to complex with the Fe(II) of the myoglobin. The pigment in these studies appears to be fully denatured and the heme exposed, as shown by the equal intensity of the pigments formed with equal concentrations of n-butyl- and benzylisocyanides. St. George and Pauling (1951) have shown that with increasing bulk of the aliphatic side chain, higher concentrations of the isonitrile were required to form heme complexes with the native pigment.

This study was undertaken solely for the purpose of establishing the structure of compounds that would react with meat components to provide a satisfactory color to meat products. The effect on flavor was not taken into consideration at this time. Furthermore, any substitute for nitrite added to a meat product would have to undergo toxicity testing to establish the safety of such a compound.

SUMMARY

A SURVEY of more than 300 compounds was carried out to find substitutes for nitrite in producing the characteristic cure color in meats. A meat emulsion slurry proved to be an effective model system for studying pigment formation. Pyridine compounds were the most effective pigment producers, depending on the nature and position of the substituent. The best pink color was formed by 3-acylpyridines. Isoquinoline, pyrazine and imidazole also formed stable pigments.

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Reference to brand or firm names does not constitute endorsement by the U.S. Dept. of Agriculture over others of a similar nature not mentioned. Precautions should be taken in handling the cyanide compounds. They are potentially hazardous.